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# A Chondrodysplasia Family Produced by Mutations in the *Diastrophic Dysplasia Sulfate Transporter* Gene: Genotype/Phenotype Correlations

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**Achondrogenesis type 1B (ACG-1B), atelosteogenesis type 2 (AO-2), and diastrophic dysplasia (DTD) are recessively inherited chondrodysplasias of decreasing severity caused by mutations in the *diastrophic dysplasia sulfate transporter* (DTDST) gene on chromosome 5. In these conditions, sulfate transport across the cell membrane is impaired which results in insufficient sulfation of cartilage proteoglycans and thus in an abnormally low sulfate content of cartilage. The severity of the phenotype correlates well with the predicted effect of the underlying *DTDST* mutations: homozygosity or compound heterozygosity for stop codons or transmembrane domain substitutions mostly result in achondrogenesis type 1B, while other structural or regulatory mutations usually result in one of the less severe phenotypes. The chondrodysplasias arising at the *DTDST* locus constitute a bone dysplasia family with recessive inheritance.** 

KEY WORDS: chondrodysplasia, achondrogenesis, atelosteogenesis, diastrophic dysplasia, sulfated proteoglycans, sulfate transporter

## INTRODUCTION

In 1994, independent findings from two laboratories pointed at defective sulfation of cartilage proteoglycans as a new pathogenetic mechanism in human chondrodysplasia. Further work has shown that three distinct chondrodysplasias of increasing severity are caused by mutations in the same gene, *DTDST* (for *diastrophic dysplasia sulfate transporter*). The gene codes for a sulfate/chloride antiporter of the cell membrane expressed in a variety of tissues, including cartilage. Here we give an account of the biochemical and molecular findings and present preliminary genotype-phenotype correlations.

### The Locus for Diastrophic Dysplasia Is a Sulfate Transporter Gene

Hästbacka et al. [1994] ended a long quest for the gene responsible for diastrophic dysplasia (DTD), a recessively inherited chondrodysplasia particularly frequent in Finland [Lamy and Maroteaux, 1960; Spranger, 1992; Horton and Hecht, 1993]. The gene had previously been mapped to the long arm of chromosome 5 [Hästbacka et al., 1992]. When finally cloned and sequenced [Hästbacka et al., 1994], the gene showed significant homology to a gene coding for a sulfate transporter from rat hepatocytes and was therefore also presumed to encode a sulfate transporter. Indeed, a defect in the rate of sulfate uptake could be demonstrated in cells from a DTD patient. Because cartilage tissue contains more sulfated proteoglycans than any other tissue, it was speculated correctly that the sulfate transport defect would affect the sulfation of proteoglycans in cartilage [Hästbacka et al., 1994].

### A Sulfation Defect in Achondrogenesis Type IB

Taking a traditional biochemical approach, we studied cells and cartilage tissue from a patient with achondrogenesis, a condition at the severe end of the spectrum of human chondrodysplasias {Fraccaro, 1952; Spranger et al., 1992; Horton and Hecht, 1993], to test for quantitative or qualitative abnormalities of the sulfated proteoglycans. The stimulus for this came from the observation that genetic deficiency of the aggrecan core protein is the cause of the lethal chicken chondrodysplasia, *nanomelic,* which resembles human achondrogenesis [Li et al., 1993]. Cartilage collagens of that patient had been examined previously by B. Steinmann, and the normal results obtained had excluded achondrogenesis type II, the variant associated with collagen II defects [Spranger et al., 1994]. The histologic changes were suggestive of achondrogenesis type IB [Borochowitz et al., 1988; van der Harten et al., 1988]. We then found that cartilage extracts and carti-

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

lage sections were devoid of materials staining metachromatically with toluidine blue, suggesting the absence of sulfated proteoglycans [Superti-Furga, 1994], and confirming a previous histochemical observation [van der Harten et al., 1988]. Subsequent studies showed that the sulfate content of ACG-1B cartilage was markedly reduced [Superti-Furga et al., 1996]. Immunoprecipitation of the small proteoglycan, decorin, from ACG-1B fibroblast cultures showed that the amount of decorin produced, its molecular weight and susceptibility to chondroitinase ABC were normal, but that tracer sulfate was not incorporated into decorin to any extent [Superti-Furga, 1994]. When sulfate metabolism of fibroblast cultures was tested, the incorporation of sulfate was reduced, and the formation of the activated sulfate metabolites, adenosine-phosphosulfate (APS), and phosphoadenosine-phosphosulfate (PAPS) was slowed. Because of the presence of free sulfate in the paper electrophoresis assay used to separate sulfate from APS and PAPS, it was erroneously concluded that the patient had a defect in the metabolic activation of sulfate [Superti-Furga, 1994]. However, subsequent experiments showed that the patient's cell homogenates were as capable of activating sulfate just as well as homogenates from control cells were.

### The Relationship Between ACG-1B and DTD

Because of the possible relationship between the two newly discovered disorders of sulfate metabolism, we tested whether sulfation of proteoglycans was indeed affected in DTD cells and, conversely, whether insufficient sulfate uptake might be responsible for impaired APS and PAPS synthesis in ACG-1B cells. The results confirmed both hypotheses: sulfate incorporation and PAPS formation was reduced in DTD cells as had been originally observed in ACG-1B cells, and sulfate uptake was impaired in ACG-1B cells as it had been shown to be in DTD cells [Superti-Furga et al., 1996]. The biochemical tests indicated that the cells were affected by essentially the same defect, i.e., impaired sulfate transport across the membrane, which lead to PAPS depletion and undersulfation of proteoglycans.

### A Third DTDST-Associated Phenotype: Atelosteogenesis Type 2 (AO-2)

In 1987, four patients originally diagnosed as having severe DTD were reclassified as a "new" chondrodysplasia, atelosteogenesis type 2, because of more severe histologic changes in cartilage [Sillence et al., 1987]. A phenotypic overlap with severe DTD was observed by several investigators [Schrander-Stumpel et al., 1994; Qureshi et al., 1994, 1995]. Older reports of lethal DTD would also be classified as AO-2 today [e.g., Briner and Brandner, 1974]. Whether a similar, neonatally lethal condition with marked fibular shortening known as *de la Chapelle dysplasia* [de la Chapelle et al., 1972] is also identical to atelosteogenesis type 2 [Whitley et al., 1987] is still unclear. The relationship between AO-2 and DTD has now been confirmed by biochemical and molecular methods: AO-2 cells show defective sulfate uptake and sulfation, and AO-2 patients carry *DTDST* mutations [Hästbacka et al., 1996; and see below].

### A Series of Mutations in the DTDST Gene

By PCR amplification, SSCP analysis, and direct sequencing several DTDST mutations have been identified in patients with ACG-1B, AO-2, and DTD, including some patients whose radiological and histological findings had been published previously [Borochowitz et al., 1988; van der Harten et al., 1988; Freisinger et al., 1994; Superti-Furga, 1994]. The mutations are shown in Table I [data from Hästbacka et al., 1994; Superti-Furga et al., 1996; Hästbacka et al., 1996; Superti-Furga and Rossi, unpublished]. Some mutations have been seen in several unrelated individuals (Table II) and may point to mutational hotspots, such as CpG dinucleotides (c558t/*R178X*, c862t/*R279W*) or short direct repeats (Δgtt 1045–1047/*ΔV340*). The "Finnish" mutation has not been identified yet but has been shown to produce reduced mRNA levels [Hästbacka et al., 1996, and Superti-Furga and Rossi, unpublished; see below].

### Is There a Correlation Between the Different DTDST Mutations and the Phenotypes?

Plotting the various combinations of mutations against the phenotype allows one to draw some preliminary conclusions. ACG-1B is associated with structural mutations predicting little or no residual activity. This is certainly the case for two patients who were compound heterozygotes for mutations each causing truncation of a major part of the DTDST protein (c559t/Δt476 and c559t2/Δa1221). Other structural mutations seen in ACG-1B patients include the nonconservative substitution or the deletion of amino acid

Table I. Overview on Known DTDST Gene Mutations[a]

| Stops, frameshifts (FS) | Missense or deletion in transmembrane domain | Missense in loops or tail | "Regulatory" (intact structure, low mRNA) |
|---|---|---|---|
| Δt476 (*FS*) | g791a (*G255E*) | c862t (*R279W*) | Finnish allele |
| c559t (*R178X*) | Δgtt (*ΔV340*) | g2060t (*G678V*) | |
| ag(1VS-1)ac (missplicing?) | a1300g (*N425D*) | c2171t (*A715V*) | |
| Δa1221 (*FS*) | t1475c (*L483P*) | | |
| Δa1751 (*FS*) | | | |
| Δa2009 (*FS*) | | | |

[a]Data from Hästbacka et al., 1994; Superti-Furga et al., 1996; Hästbacka et al., 1996; Superti-Furga and Rossi, unpublished. Nucleotide and amino acid numbering follows Hästbacka et al., 1994.

Table II. DTDST Gene Mutations Observed in Multiple Unrelated Patients

| Mutation | Observed on | Ethnic / geographic origin |
|---|---|---|
| c559t (*R178X*) | 3 ACG-1B chromosomes, 2 AO-2 chromosomes, 1DTD chromosome | 2 France, 2 "Europe," 1 "Hispanic" (USA), 1 Netherlands |
| c862t (*R279W*) | 3 AO-2 chromosomes, 5 DTD chromosomes | 3 Netherlands, 5 not known |
| Δgtt 1045-1047 (*ΔV340*) | 3 ACG-1B chromosomes | 1 Turkish, 1 French, 1 "Hispanic" (USA) |
| Δa1751 (*FS*) | 1 ACG-1B chromosome, 2 AO-2 chromosomes, 3 DTD chromosomes | 2 Netherlands, 1 Germany, 1 France, 2 not known |

residues within one of the transmembrane domains of DTDST, and the substitution of a conserved glycine residue within the cytoplasmic tail of DTDST. The latter mutation is more difficult to interpret as the function of this portion of DTDST is not known.

Structural mutations have never been found on both chromosomes of DTD patient. DTD patients appear to have at least one allele with an intact coding region (except for a neutral amino acid substitution, T689S, found also in controls) associated with reduced mRNA levels [Hästbacka et al., 1996, Superti-Furga and Rossi, unpublished]. The underlying genomic mutation has not yet been found. This allele probably has some residual activity and, though pathogenic, results in the milder DTD phenotype even when compounded with a structural mutation on the other chromosome.

The genotypes of AO-2 patients are more complex to interpret. Two AO-2 patients have been shown to carry structural mutations on both DTD genes [Hästbacka et al., 1996]. In each case, one mutation would be expected to abolish activity (g790a/G255E, which is a non-conservative substitution in a transmembrane domain, and Δa1751/*frameshift*, which truncates the protein), while the second mutation in each patient may have less dramatic consequences: c862t/*R279W* predicts a non-conservative substitution in an extracellular loop, while c2171t/A715V is located very close to the carboxy-terminus of the protein, the function of which is not known [Hästbacka et al., 1994]. Two AO-2 patients have been identified who are heterozygous for the c862t/*R279W* mutation and who express only low levels of the other allele based on RT-PCR analysis (and thus possibly carry one copy of the "Finnish" mutation) [Hästbacka et al., 1996; Superti-Furga and Rossi, unpublished]. This same constellation has been seen also in one diagnosed as DTD [Superti-Furga and Rossi, unpublished]. Two further AO-2 patients carry the c862t/*R279W* mutation compounded with yet another, still uncharacterized mutation [Rossi and Superti-Furga, unpublished]. Altogether, the data confirm that AO-2 is a severe variant of DTD.

## Prenatal Diagnosis of ACG-1B, AO-2, and DTD

In several instances, ACG-1B, AO-2, or DTD fetuses have been diagnosed *in utero* by the ultrasonographic demonstration of short and malformed limbs. While ACG-1B can be distinguished from other achondrogenesis variants and from AO-2 by the typical combination of radiological and histological changes [van der Harten et al., 1988; Borochowitz et al., 1988, Superti-Furga, 1994], it is not easy to distinguish between AO-2 and severe DTD by either method, and such a distinction in a fetus may be somewhat arbitrary. Prenatal diagnosis can now be made by mutation analysis of chorionic villus DNA, provided the index case has been fully characterized. In other cases, analysis of closely linked markers can be used [Hästbacka et al., 1993]. Whether sulfation analysis on CVS might be used for diagnostic purposes remains to be investigated.

## Sulfated Proteoglycans and Chondrodysplasias: Further Research Lines

The study of proteoglycans synthesized by ACG-1B chondrocytes cultured in alginate has shown that defective sulfation does not cause a generalized defect in proteoglycan synthesis: the cartilage proteoglycans are undersulfated but otherwise normal [Rossi et al., submitted]. This raises the question of how defective sulfation produces the severe histologic changes and skeletal growth defect in ACG1B, AO-2, and DTD. Probably, the absence of sulfate groups on the glycosaminoglycan side chains disrupts some interaction between the GAG chains and other yet unidentified ligands, leading to a disturbance in the assembly of cartilage matrix. Identification of these ligand(s) and of their interaction with sulfated GAGs would reveal new aspects of cartilage matrix assembly.

It is predictable that mutations in other genes pertaining to the synthesis of sulfated proteoglycans may cause chondrodysplasias. The gene for the sulfate activating enzyme, ATP sulfurylase/APS kinase, which is responsible for the mouse chondrodysplasia mutant *brachymorphic*, is a candidate [Sugahara and Schwartz, 1982]. An equally good candidate is the gene coding for the core protein of the major cartilage proteoglycan, aggrecan, mutations in which cause the *nanomelic* chondrodysplasia in chicken [Li et al., 1993] and the *cartilage matrix-deficient* chondrodysplasia in the mouse [Watanabe et al., 1994].

## The DTDST Chondrodysplasia Family

The concept of bone dysplasia "families" had been put forward by Spranger in 1987 [Spranger, 1988] and validated by the subsequent identification of "families" of chondrodysplasias of different severity caused by mutations in the same gene, such as the collagen II [Spranger et al., 1994] and the FGFR-3 chondrodysplasia families [Tavormina et al., 1995; Bellus et al., 1995;

Superti-Furga et al., 1995]. The results presented above indicate that achondrogenesis type 1B, atelosteogenesis type 2, and diastrophic dysplasia constitute a separate chondrodysplasia family. While the collagen II and FGFR-3 chondrodysplasias are caused by dominant mutations, DTDST chondrodysplasias are recessively inherited.

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